C型ナトリウム利尿ペプチドの体内分布と血管内皮由来局所調節因子としての意義に関する研究(Dissertation_全文)

AUTHOR(S):

Komatsu, Yasato

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C-TYPE NATRIURETIC PEPTIDE (CNP) IN RATS AND HUMANS

Yasato Komatsu, Kazuwa Nakao, Shin-ichi Suga, Yoshihiro Ogawa, Masashi Mukoyama, Hiroshi Arai, Gotaro Shirakami, Kiminori Hosoda, Osamu Nakagawa, Norio Hama, Ichiro Kishimoto and Hiroo Imura.

Second Division, Department of Medicine, Kyoto University School of Medicine, Kyoto 606, Japan.

ABSTRACT: We have established a specific radioimmunoassay (RIA) for C-type natriuretic peptide (CNP), the third member of the natriuretic peptide family, and have elucidated its tissue distribution and molecular form. In rats, high concentrations of CNP-like immunoreactivity (-LI) were detected in the anterior lobe (19.8 ± 8.6 pmol/g) and neurointermediate lobe (4.64 ± 0.74 pmol/g) of the pituitary gland. CNP-LI was present throughout the brain with its high concentrations in the hypothalamus and cerebellum. Small amounts of CNP-LI were also detected in the lower part of gastrointestinal tract and the kidney. However, no significant amount of CNP-LI was present in other organs including the heart. Considerable amounts of CNP-LI were detected throughout the human brain. High performance-gel permeation chromatography coupled with the RIA detected two peaks of CNP-LI in the rat brain; CNP and presumably its N-terminally elongated form with 53 amino-acid residues, CNP-53. These findings indicate that the tissue distribution and processing pattern of CNP are clearly different from those of atrial natriuretic peptide and brain natriuretic peptide and suggest possible roles of CNP as a neurotransmitter or neuromodulator rather than as a cardiac hormone.

Since the discovery of atrial natriuretic peptide (ANP) in the heart and subsequently in the brain, it has been implicated in body fluid homeostasis and blood pressure control as a neuropeptide as well as a cardiac hormone (1-3). A second natriuretic peptide, originally isolated from the porcine brain and thereafter named brain natriuretic peptide (BNP), also occurs in the heart (4-9), and has been reported to evoke central and peripheral actions similar to those of ANP (4,10-12). It is now recognized that ANP and BNP are members of a natriuretic peptide family, thus regulating various components of cardiovascular homeostasis. We have demonstrated that ANP is mainly synthesized in and secreted from the atrium, while BNP is mainly by the ventricle in rats and humans (6-9). Recently, a third member of the natriuretic peptide family designated as C-type natriuretic peptide (CNP) has been identified in the porcine brain, which has 22 amino-acid residues (13). CNP has a ring structure formed by an intramolecular disulfide bond, which is conserved in both ANP and BNP, but uniquely it lacks the C-terminal extension from the ring structure. More recently, an N-terminally elongated form with 53 amino-acid residues, named CNP-53, has been isolated from the porcine brain (14). In the present study, we have developed a specific radioimmunoassay (RIA) for CNP and have investigated its tissue distribution and molecular form in rats. We have also examined the distribution of CNP in the human brain.

MATERIALS AND METHODS

Peptides: CNP, [Tyr^0]-CNP, porcine BNP, rat BNP and human BNP were synthesized by the solid phase method. α-ANP was purchased from Peptide Institute Inc., Minoh, Japan.

Conjugation and immunization of CNP: CNP (3.0 mg) was conjugated to bovine thyroglobulin (15.0 mg, Sigma Chemical Co., St. Louis, MO, USA) using the carbodiimide coupling procedure as previously described (15). Immunization was performed using BALB/c mice, as we previously reported (15).

Iodination: [Tyr^0]-CNP was radioiodinated by the chloramine T method (15). The specific activity of [^{125}I][Tyr^0]-CNP ranged from 400 to 700 μCi/μg.

RIA for CNP: RIA for CNP was performed following the method of RIA for ANP (15). The RIA incubation mixture consisted of 50 μl of standard or samples, 50 μl of a final 1:20,000 dilution of an antiserum (C-5), 50 μl of [^{125}I][Tyr^0]-CNP (approximately 10,000 cpm) and 100 μl of the standard buffer. The antiserum and standard or sample were incubated for 24 hours at 4°C, after which [^{125}I][Tyr^0]-CNP was added and incubated for another 10 hours at 4°C. Bound and free ligands were separated by the dextran-coated charcoal method (15).

Tissues and extraction procedure: Tissues were obtained from male Slc: Wistar rats (n=4), weighing 250-300 g (Shizuoka Animal Center, Shizuoka, Japan) immediately after decapitation and were dissected as previously described (15,16). Blocks of discrete regions of a human brain were obtained at autopsy from a patient without neurological complications. Informed consent was obtained from the patient's family. The dissected tissues were extracted as previously reported (15,16).

High performance-gel permeation chromatography (HP-GPC): HP-GPC was performed on a TSK-GEL G2000 SW column (7.5 x 600 mm) (Toyo Soda, Tokyo, Japan) (15,16).

RESULTS

RIA for CNP: A typical standard curve of CNP in the RIA using an antiserum against CNP (C-5) is shown in Fig. 1. The minimal detectable quantity in the RIA was 2.0 fmol/tube and the 50 % binding intercept was 30 fmol/tube. The cross-reactivities of α-ANP, porcine BNP, rat BNP, human BNP were 0.2 %, 14 %, <0.01 %, <0.01 %, respectively. Intra- and inter-assay coefficients of variation were 8.7 % (n=9) and 9.1 % (n=8), respectively.

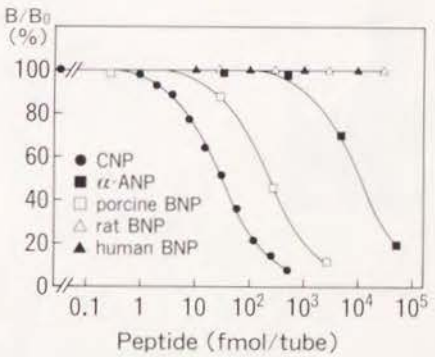

Fig. 1 A representative standard curve of CNP (closed circles) and dilution curves of α-ANP, porcine BNP, rat BNP and human BNP.

Address correspondence to: Kazuwa Nakao, M.D., Ph.D., Second Division, Department of Medicine, Kyoto University School of Medicine, 54 Shogoin Kawahara-cho, Sakyo-ku 606, Japan. Phone No. 075 (751) 3170
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Tissue distribution of CNP in rats: Serial dilution curves of extracts of the rat whole brain and pituitary were parallel to the CNP standard curve and representatives of dilution curves are shown in Fig. 2. Table 1 gives the distribution of CNP-like immunoreactivity (-LI) in the rat tissues. The highest concentrations of CNP-LI were detected in the anterior lobe (19.8 ± 8.6 pmol/g) and neurointermediate lobe (4.64 ± 0.74 pmol/g) of the pituitary gland. In the brain, CNP-LI was widely present with its high concentrations in the hypothalamus and cerebellum. Small amounts of CNP-LI were also detected in the kidney, ileum and colon. But no detectable amount of CNP-LI was present in other tissues, including the ventricle of the heart, liver, spleen, lung, testis, parotid gland, thymus, stomach, duodenum, jejunum, cecum and adrenal gland (less than 0.17 pmol/g). Although CNP-LI was detectable in the atrium (39.0 ± 4.4 pmol/g), this CNP-LI could be attributed to the cross-reactivity of ANP in our RIA.

CNP in the human brain: CNP-LI levels were determined in discrete regions of the human brain (Table 2). Serial dilution curve of each sample was also parallel to the CNP standard curve. CNP-LI was widely present in the human brain. High concentrations of CNP-LI were detected in the hypothalamus and midbrain.

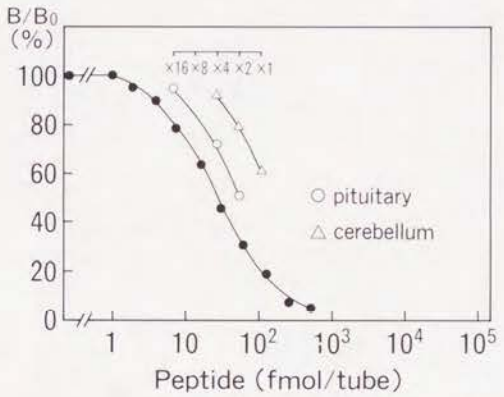

Fig. 2 A standard curve of CNP (closed circles) and dilution curves of the extracts from the rat pituitary and cerebellum.

Table 1 Regional distribution of CNP-like immunoreactivity (-LI) in rats

Tissues	CNP-LI
Brain	
Olfactory bulb	1.39 ± 0.11
Cerebral cortex	0.69 ± 0.12
Hippocampus	1.20 ± 0.15
Striatum	0.74 ± 0.15
Thalamus	1.79 ± 0.42
Hypothalamus	2.35 ± 0.29
Septum	1.74 ± 0.31
Midbrain	1.32 ± 0.29
Pons	0.69 ± 0.13
Medulla oblongata	1.33 ± 0.11
Cerebellum	3.07 ± 0.53
Pituitary gland	
Anterior lobe	19.8 ± 8.6
Neurointermediate lobe	4.64 ± 0.74
Kidney	0.66 ± 0.12
Ileum	0.46 ± 0.14
Colon	0.31 ± 0.06

Values are mean ± SE (pmol/g wet tissue)

Table 2 Regional distribution of CNP-like immunoreactivity (-LI) in human brain

Brain region	CNP-LI
Cerebral cortex	0.61
Thalamus	2.90
Hypothalamus	4.02
Midbrain	3.28
Pons	< 0.40
Medulla oblongata	2.78
Cerebellum (cortex)	0.85

(pmol/g wet tissue)

Molecular form: Figure 3 shows HP-GPC profile of the extract from the rat whole brain. Two peaks with CNP-LI were observed. One peak eluted at the position of 2K-3K daltons, corresponding to synthetic CNP and the other peak eluted at the position with apparent molecular weight of 5K-6K daltons.

DISCUSSION

In the present study, we have established a specific RIA for CNP and have elucidated its tissue distribution and molecular form. Using an antiserum raised against porcine CNP, we could detect CNP-LI in rats and humans, suggesting that the structure of CNP is conserved among species. This notion is supported by the recent analysis of rat CNP complementary DNA (17) and our unpublished study of the human CNP gene showing that the C-terminal 22-amino acid sequences of rat and human preproCNP are identical to porcine CNP. This result contrasts with the previous reports that the RIA for porcine BNP cannot detect BNP-LI in rats and humans and that the structures of BNPs are divergent among species (4-9).

In rats, we have detected the highest concentration of CNP-LI in the pituitary gland, where no significant amounts of ANP-LI and BNP-LI were reported (8,16). Furthermore, CNP-LI is present in

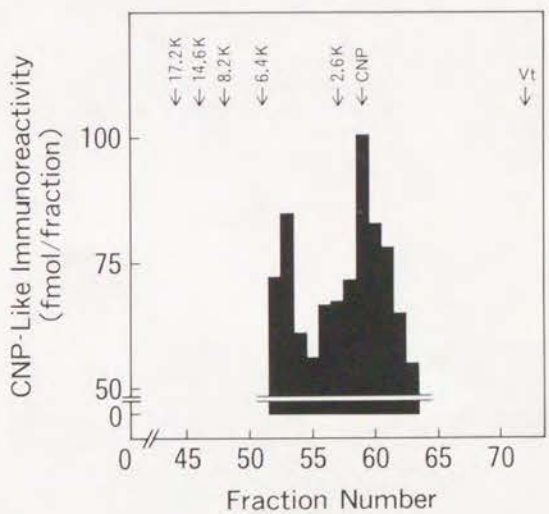

Fig. 3 A gel filtration profile of CNP-LI in the rat brain on a TSK-GEL G2000 SW column. Arrows indicate the elution positions of synthetic CNP, a series of myoglobins of a polypeptide molecular kit (Pharmacia Fine Chemicals, Uppsala, Sweden) and total volume (Vt).

the hypothalamus at a relatively high concentration. These results suggest the possibility that CNP is a neuroendocrine modulator responsible for the hypothalamo-pituitary function.

The CNP-LI levels are comparable to the ANP-LI levels throughout the rat brain, however, the distribution of CNP-LI is clearly different from that of ANP-LI (16). We previously reported no significant amount of BNP-LI is detectable in discrete regions of the rat brain (8). Of note is the high concentration of CNP-LI in the cerebellum, where no detectable amounts of ANP-LI and BNP-LI were present (8,16).

In the present study, although we detected CNP-LI in the atrium, it could be explained by the cross-reactivity of ANP in our RIA. And no significant amount of CNP-LI was detected in the rat plasma (data not shown). These results are consistent with the recent finding that CNP messenger RNA is hardly detectable in the rat atrium and ventricle under the same condition as is detected in the brain (17). These results make a striking contrast to the previous observation that the heart is the major source of ANP and BNP (8,15), suggesting that CNP works mainly as a neuropeptide, while ANP and BNP mainly as cardiac hormones.

We have detected considerable amounts of CNP-LI in discrete regions of the human brain and its concentration and distribution are similar to those in the rat brain, suggesting possible roles of CNP in the human brain.

HP-GPC study demonstrates that CNP-LI in the rat brain is composed of two components; one comigrating with synthetic CNP and the other with apparent molecular weight of 5K-6K daltons. No immunoreactivity was detectable at the elution position of the CNP precursor. In the porcine brain, CNP has two molecular forms; CNP with 22 amino acids and CNP-53 (13,14). Furthermore, it has been reported that the C-terminal 53-residue peptide of the rat preproCNP is identical to porcine CNP-53 (17). Together with our unpublished observation that the HP-GPC profile of CNP-LI in the porcine brain is almost identical to that in the rat brain, it is likely that both CNP and CNP-53 are endogenous forms in the rat brain, as in the porcine brain (13,14). On the contrary, we previously reported that the major component of ANP-LI in the rat brain is composed of the ANP precursor (γ-ANP) and 2 kinds of N-terminally deleted forms of α-ANP; α-ANP[4-28], and α-ANP[5-28] (18). These findings indicate that CNP has a distinct processing pattern from ANP in the rat brain.

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